Appendix: Nano Silver Toxicology Information and References

The biocidal potency of silver is related to the potential for release of silver ions (Klaine et al., 2008, Nowack et al., 2011; Xiu et al., 2012). Particle properties that contribute to direct toxicity of silver nanoparticles such as size, shape, surface coating and surface charge are also likely to affect toxicity indirectly through mechanisms that influence the rate, extent, location and/or timing of silver ion release (Xiu et al., 2012). The high surface area per mass of the nanoparticle provides the opportunity for closer interaction with the surrounding materials and environment. The nanoparticles may also have different physico-chemical properties and biological activity as compared to regular/bulk silver (Burrell, 2003; Wijnhoven et al., 2009).

Toxicokinetics – systemic availability

In rats, oral administration of 60 nm silver nanoparticles at doses 30-1000 mg/kg/day for 28 days resulted in a dose dependent accumulation of silver content in blood, stomach, brain, liver, kidneys, lungs and testes indicating systemic distribution. Kidney accumulation showed gender differences, with females accumulating twice as much compared to males at all doses (Kim et al., 2008).

In an oral gavage study, rats were administered silver nanoparticles (10-18 nm) in aqueous solution of 11.5 mg/mL polyvinyl pyrrolidone (PVP) at 10 mL/kg bw, twice daily for 28-days and the highest silver concentrations were detected in small intestines, kidneys and the liver with lower amounts in the lungs and brain (Loeschner et al., 2011). The excretion of silver in faeces was 63% (+/-23%) of the daily dose and excretion via urine was low (<0.1%).

In a recent study, male rats were fed for 28 days either with a polymer coated silver nanoparticles, uncoated nanoparticles or a silver nitrate solution (details not available) to measure silver levels in tissues (Lubick, 2012). The total silver levels were ten times higher in the livers and spleens of rats fed with silver nitrate compared to nanoparticles indicating that rats absorb silver ions more readily compared to nanoparticles. This article also reported that silver ion treated rats had silver nanoparticles in their tissues due to precipitation of silver as insoluble salts that form nanoparticles. These findings suggested that silver changes form between ions and nanoparticles inside animals regardless of the form ingested.

Application of silver nanoparticles (20 and 50 nm) suspensions to skin of pigs at doses ranging from 0.34 to 34.0 µg/mL for 14 days resulted in the presence of 50 nm particles within the superficial layers of the stratum corneum and 20 nm particles in the top layer of the stratum corneum (Samberg et al., 2010).

In patients received treatment with dressings containing nanocrystalline silver (~15 nm particles) for a period of 28 days or less (depending on clinical requirement), the maximum serum silver levels increased with exposure to silver dressing but returned to the levels at initial assessment time, 3 months after treatment was discontinued. No biochemical indicators of toxicity were observed (Vlachou et al., 2007). In an in vitro study, absorption of polyvinyl pyrrolidone coated silver particles (~9 – 50 nm particles dispersed in synthetic sweat) through damaged human abdominal skin is greater (0.62 +/- 0.2 ng/cm^2) than through normal healthy skin, but the absorption was very low (Larese et al., 2009).

Rats exposed to aerosolized silver nanoparticles (18-19 nm) for 90 days by inhalation (49-515 µg/ m^3) also showed systemic distribution with significant dose-dependent increases in silver concentrations in the blood, liver, olfactory bulb, brain and kidneys. Concentrations were similar in males and females except in kidneys where the female kidneys accumulated two or three times more silver (Sung et al., 2009).

Mice repeatedly exposed to 5±2 nm silver particles by inhalation at 3.3 mg/m3 for 4 hours/day, 5 days/week for 2 weeks retained 4% of the total silver dose administered (803 µg/g lung dry weight in the pulmonary region). This rapidly reduced to 1.3% of the administered dose by the third week following last exposure. Similar concentrations were found in the bronchial alveolar lavage (BAL) fluid providing evidence of silver dissolution in the lungs. Silver concentration in the heart, liver and brain in the same mice was below the detection limit (Stebounova et al., 2011).

Silver was rapidly cleared from the lungs of rats and entered systemic pathways following inhalation (7.2 µg of 15 nm particles). By day 7, the lung burden was 4% of the initial lung burden measured immediately after exposure. Lower concentrations of silver were found in the liver, kidney, spleen, brain and heart and relatively higher concentrations were found in nasal cavities (posterior portion) and lung-associated lymph nodes (Takenaka et al., 2001).

Single subcutaneous injections of silver nanoparticles (50-90 nm) at 62.8 mg/kg bw in rats showed that >99.8% of particles remained at the injection sites or were excreted. The maximal content of silver nanoparticles (determined by transmission electron microscopy and scanning electron microscopy images) in the organs was 0.15% of the total injected dose. The

percentage of silver content in different organs increased up to 12 weeks and then remained consistent up to 24 weeks. Kidney, liver, spleen, brain, lung and blood showed significantly higher accumulation of silver while some increase was also seen in heart, uterus, ovaries, adrenal glands and femur. Particles could also cross the blood brain barrier, accumulate in the brain and cause neuronal degeneration. The silver content in faeces was significantly higher than urine in the first 8 weeks indicating that excretion was mostly through the faeces (Tang et al., 2009).

Intravenous administration of 24-28 µg of silver nanoparticles (20, 80 and 110 nm particles) for up to 5 consecutive days in rats led to distribution of particles to many organs and accumulation in lung, liver and spleen. The smaller particles distributed mainly to the liver and the larger particles mainly to the spleen (Lankveld et al., 2010). Similar experiments in mice using 12 nm silver particles radiolabelled with iodine-125 also showed predominant accumulation of particles in the spleen and liver at 24 hours post exposure (Chrastina et al., 2010).

Acute toxicity

In an acute oral toxicity study conducted according to the Organisation for Economic Co-operation and Development Test Guidelines (OECD TG) 423 in rats with 10 nm silver particles, there were no mortalities or signs of toxicity up to 2000 mg/kg bw (oral LD50 >2000 mg/kg bw) (Kim et al., 2012). In another acute oral toxicity study (OECD TG 425 – up and down procedure) pure silver nanoparticles (10-20 nm, silver ions less than 0.04%) caused no mortalities or toxicity signs in mice at a limit dose of 5000 mg/kg bw (LD50 >5000 mg/kg bw) (Maneewattanapinyo et al., 2011). In an oral gavage study, mice dosed with 2.5 g of 13 nm silver particles showed effects in the liver, intestine, heart and spleen 3 days after treatment. There were no mortalities reported (Cha et al., 2008).

In an acute dermal toxicity study (OECD TG 402) in rats with silver nanoparticles (average size = 10 nm), there were no mortalities, signs of toxicity or gross pathological findings at necropsy at 2000 mg/kg bw, indicating dermal LD50 as >2000 mg/kg bw (Kim et al., 2012). In another study (OECD TG 434 – fixed dose procedure) guinea-pigs received silver nanoparticles (10-20 nm) in suspension (2 mL) on 7x10 cm^2 shaved skin area and left covered for 24 h and showed no toxicity or mortalities at doses of 50 or 100,000 ppm (Maneewattanapinyo et al., 2011).

There were no mortalities, significant body weight changes or significant changes in lung function tests in rats exposed to 20 nm silver particles at doses of 76, 135 and 750 µg/m^3 for 4 hours in an acute inhalation toxicity study (OECD 403). The study indicated an LC50 > 750 µg/m^3 (3.08 x 106 particles/cm^3) which was the highest dose tested (Sung et al., 2011). Considering the low dose tested in this study it is not possible to derive a meaningful LC50 value for acute inhalation toxicity.

Eye and skin irritation

There are only two references available on skin and eye irritation of silver nanoparticles. Silver nanoparticles with average particle size of 10 nm produced no eye irritation (OECD TG 405) or skin irritation (OECD TG 404) in New Zealand rabbits (Kim et al., 2012). In studies conducted according to the OECD TG, Maneewattanapinyo et al. (2011) reported no eye or skin irritation in guinea pigs with 10 - 20 nm particles (99.96% pure with only 0.04% Ag ions).

Skin sensitisation

There is only one reference available on skin sensitisation of silver nanoparticles (average size 10 nm) (Kim et al., 2012). In a guinea-pig maximization test (OECD TG 406), one animal (out of 20) showed discrete and patchy erythema, 24 and 48 h after challenge, respectively. The test material was reported as a weak skin sensitiser.

Repeated dose toxicity

Oral

Four different sizes of uncoated silver particles (22, 42, 71 and 323 nm) were administered at 1 mg/kg bw/day in the diet of mice for 14 days. Silver from smaller particles (22-71 nm) translocated to blood, accumulated in various organs (brain, lung, liver, kidney, and testis) and induced inflammatory responses. Silver was not detected in tissues of mice treated with larger sized particles (323 nm). Body weight or organ/body weight ratios were not affected in any treated group. In an extension of

the same study, 42 nm silver particles were administered in the diet for 28 days at three different doses (0.25, 0.50 or 1 mg/kg bw). Significant increases in alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were seen in the highest dose group of 1 mg/kg bw/day, indicating some hepatotoxicity. Increases in cytokines were seen at all doses, with some pro-inflammatory cytokines showing dose dependent increases. Some histopathological changes were seen in the kidney at the highest dose, but were absent in the liver and intestines, which was attributed to the low dose tested. While histopathological evidence was poor, inflammatory responses were seen at all doses and a NOAEL was not reported (Park et al., 2010). Based on hepatotoxicity and histopathological changes in kidneys at the highest dose, a NOAEL of 0.50 mg/kg bw/d can be established.

In a 28 day repeat dose oral toxicity study (OECD TG 407), rats exposed to 30, 300 or 1000 mg/kg bw doses of 60 nm silver particles showed presence of silver nanoparticles in the intestinal tissues at 300 mg/kg bw or higher and changes in the histological structure and properties of the muco-substances in the intestinal mucosa. A dose-dependent accumulation of silver nanoparticles was reported in parts of both small and large intestines (Jeong et al., 2010). Adverse health effects related to dose levels tested were not reported.

In a 28 day repeat dose oral gavage study (OECD TG 407), mice exposed to 30, 300 or 1000 mg/kg bw doses of 60 nm silver particles in carboxymethyl cellulose showed no significant changes in body weight gain. However ALP and cholesterol values were significantly increased in the blood of the groups treated with 300 or 1000 mg/kg bw suggesting possible liver damage. All treated groups showed significant dose-dependent increase of silver in blood, stomach, brain, liver, kidney, lungs and testes, with two fold higher concentrations in female kidneys compared to male kidneys (Kim et al., 2008). Apart from increased levels of silver in tissues, no adverse effects were reported at the lowest dose of 30 mg/kg bw and therefore, this dose can be considered as a NOAEL.

In a 90-day repeat dose oral study (OECD TG 408) rats were exposed to 30, 125 or 500 mg/kg bw of 60 nm silver particles in carboxymethyl cellulose. Accumulation of silver nanoparticles was reported in all regions of the kidneys with a clear gender difference in accumulation in kidneys, urinary bladder and adrenal glands. Female kidneys showed a 2 fold higher concentration compared to male kidneys (Kim et al., 2009). Treatment-related effects were not reported to establish a NOAEL.

In another 90 day oral study conducted in rats (OECD TG 408) using similar particle sizes and doses as in the Kim et al. (2009) study, a NOAEL of 30 mg/kg bw/day was established based on decreased kidney weight and some non-adverse hematological effects in low dose female rats. The LOAEL was 125 mg/kg bw/day based on dose related bile duct hyperplasia and increased foci in the liver and increased pigmentation of villi in the intestines (Kim et al., 2010).

Dermal

There are no repeat dose dermal toxicity studies in animals. Two studies reported the use of silver containing wound dressings in humans and one study reported the use of silver containing dressing in mice following thermal injury.

Repeated uses of Acticoat dressing containing 15 nm silver nanoparticles for 6 days (new dressings put on days 1 and 4) on wounds caused argyria like symptoms and elevated liver enzymes suggesting hepatoxicity in a person with burns indicating absorption through damaged skin. Facial discolouration and liver effects were reversed with the removal of this treatment. (Trop et al., 2006).

A study was conducted with 30 patients with burns (median total postoperative wound size of 12% of the total body surface area) that required use of acticoat dressings over a period of 28 days or less. The patients showed no haematological or biochemical indicators of toxicity associated with silver absorption from the dressings (Vlachou et al., 2007).

Mice treated daily with silver nanoparticles based dressing following thermal injury were observed over a period of 25 days or until wound closure. The 4 x 3 cm^2 dressing contained 0.48 mg of 15 nm silver particles. The authors reported reduced inflammation and modulation of fibrogenic cytokines leading to shorter healing times with no adverse effects (Tian et al., 2007).

Inhalation

Mice inhaling 5 nm silver particles at a dose of 3.3 mg/m^3, 4 hours/day for 2 weeks (5 days/week) showed minimal pulmonary inflammation or cytotoxicity (Stebounova et al., 2011). No significant toxicological effects were reported in rats exposed to silver nanoparticles (~15 nm) via inhalation for 28 days at various particle concentrations ranging from 1.2x104 to 1.2x106 particles/cm^3 (equivalent to 0.48 to 61.24 $\mu g/m^3$) for 6 hours/day, 5 days/week for 4 weeks (Ji et al., 2007).

In a study to investigate the effects of silver nanoparticles on the brain, mice were exposed to 25 nm silver particles (1.91x107 particles/cm^3), 6 hours/day for 14 days. Silver nanoparticles did not induce any structural changes in the mouse brain; however some genes in the brain were identified as responsive to exposure indicating the potential for neurotoxicity and immunotoxicity (Lee et al., 2009).

No significant dose related changes were noted in body weight, organ weight and haematology values in rats from 90 days inhalation exposure to ~20 nm silver particles at doses of 48.94 µg/m^3 (6.64x105 particles/cm^3), 133.19 µg/m^3 (1.43x106 particles/cm^3) or 514.78 µg/m^3 (2.85x106 particles/cm^3). Lungs and liver were determined to be the main target organs. Dose dependent decrease in tidal volume, minute volume and peak inspiration flows accompanied by histopathological changes (mixed cell infiltrate and chronic alveolar inflammation) were seen in the lungs and a higher incidence of bile-duct hyperplasia and perivascular infiltrate in the liver were seen at the highest dose (514.78 µg/m^3). No significant differences were seen in kidney function tests, except a significantly higher percentage of erythrocyte aggregation in the high dose females compared to controls. This study was published in two journals (Sung et al., 2008 and 2009). The authors determined a NOAEC of 100 µg/m^3 based on significant bile-duct hyperplasia, chronic alveolar inflammation and macrophage accumulation in lungs of males and females and erythrocyte aggregation in females at the highest dose of 514.78µg/m^3.

Another 12-week inhalation toxicity study conducted in rats with 14-15 nm particles confirmed the lung function changes, pathological findings in the lung and the NOAEC of 100 µg/m^3 established in the previous study (Song et al., 2012). The 2012 study reported a NOAEC of 117 µg/m^3 for rats after a 12-week recovery period. Using the rat NOAEC of 100 µg/m^3, the authors have calculated human NOAECs of 47 µg/m^3 (with heavy exercise) and 23 µg/m^3 (with light exercise) using the Multiple Path Particle Dosimetry Model (MPPD) (details of calculation not available).

Human evidence

In a health surveillance case study, silver levels in blood and urine were tested in two male workers from a manufacturing facility which produces silver nanoparticles (20-30 nm) using a completely closed system from the reactor to the collector (Lee et al., 2011). Nanoparticles released from the collector into the workplace air were minimal and the exposure levels were 0.35 and 1.35 µg silver/m^3. For the two workers working in the manufacturing facility for 7 years, the levels of silver in blood were 0.034 and 0.0135 µg/dL and the levels of silver in urine were 0.043 µg/dL for one worker and not detected in the other. Blood biochemistry and haematology results showed no adverse findings.

Reproductive and developmental toxicity

There are no reproductive or developmental toxicity studies available on silver nanoparticles. However, there are two relevant rodent studies; one reported the effects on foetuses following a single intraperitoneal administration into pregnant rats (Mahabady, 2012) and the other reported distribution and accumulation of nanoparticles following intravenous injection into pregnant mice (Austin et al., 2011). Mahabady (2012) investigated teratogenicity of nanosilver (particle size not reported) in rat foetuses following a single intraperitoneal administration of 0.4 or 0.8 mg nanosilver/kg bw into pregnant rats on gestation days (GD) 8 or 9. The foetuses collected on gestation day 20 (of rats treated on GD 8 with both doses and from the highest dose group on GD 9) had significantly reduced weights and lengths. Lower placenta weights, volumes and widths compared to the control group were reported for all treated groups. There were no macroscopic anomalies or skeletal effects in rat foetuses. Presence or absence of maternal effects was not reported.

Distribution of silver nanoparticles (~50 nm) in pregnant mice and developing embryos was investigated following intravenous injection into pregnant mice on GD 7, 8 and 9 at doses of 0, 35 or 66 µg silver per mouse (Austin et al., 2011). Silver concentrations (including nanoparticles and aggregates) in all organs/tissues, including embryos, were significantly increased in treated groups compared to the control group. The highest concentrations were reported in the liver, spleen and visceral yolk sac. The lowest concentrations were in embryos and maternal brain tissues. Both dose levels showed similar concentrations in tissues (no dose response) indicating that a tissue saturation limit was not reached at either dose tested.

In a 28-day oral gavage study, dose-dependent accumulation of silver in rat testes was reported from doses of 30 to 1000 mg/kg bw/day (Kim et al., 2008).

Genotoxicity

Using silver nanoparticles of 5 nm, Li et al. (2012) conducted a reverse mutation assay (Ames) using five Salmonella strains (OECD TG 471) and an in vitro micronucleus assay using human lymphoblastoid TK6 cells (OECD TG 487). There were no increases in mutant frequency in all five Salmonella strains tested at doses of 2.4 to 38.4 µg per plate. In human lymphoblastoid cells the micronucleus frequency was increased in a dose-dependent manner with 10 – 30 µg silver nanoparticles/mL. The authors reported the statistically significant positive result as a weak response according to their criteria. The negative result in the Ames assay was suggested as due to the inability of the nanomaterials to penetrate the bacterial cell wall or due to the insensitivity of the test strains to oxidative DNA damage. The in vitro micronucleus assay was considered a more appropriate test compared to the Ames test for evaluating genotoxicity of nanomaterials (Li et al., 2012).

Genotoxicity of 5 - 45 nm silver particles (average 19.7 nm) was tested using a Comet assay in human peripheral blood cells and in mice (Tavares et al., 2012). The in vitro Comet assay showed DNA damage at all doses (10, 25 and 50 µg/mL) in the initial hour of exposure and only at the two high doses after the first hour, possibly through the generation of reactive oxygen species. The DNA damage decreased with time indicating that the DNA may have been restored by the repair system. The in vivo study conducted to compare/confirm the in-vitro findings reported negative results suggesting activation of a cellular antioxidant network preventing DNA damage in mice (Tavares et al., 2012).

There are some in vitro studies demonstrating DNA damage in mammalian cells with exposure to silver nanoparticles, although the mode of action is not clear. Decrease in DNA content with no effect on mitochondria (Cha et al., 2008), concentration dependent increase in DNA-strand breaks in human testicular embryonic carcinoma cells (Asare et al., 2012), damage to DNA by increase in reactive oxygen species (ROS) and hampering the enzymes required for DNA repair (Asharani, 2009), binding closely with DNA in the presence of a detergent (Chi et al., 2009), upregulation of DNA damage repair proteins including P53 by coated and uncoated particles (Ahamed et al., 2008) and directly influencing DNA replication by binding to DNA (Yang et al., 2009) have been reported.

In one in vitro study, dose dependent inhibition of murine embryonic stem cell differentiation by silver nanoparticles of 20, 80 and 113 nm was reported (Park et al., 2011). The highest inhibition was reported for 20 nm particles, however, this inhibition was not as potent as with ionic silver. In the same research, 80 and 113 nm particles showed increased gene mutation frequencies (without statistical significance) in mouse embryonic fibroblasts at doses of 0.1 – 100 µg/mL following 16 hours of incubation. Gene mutation frequency was not increased by 20 nm particles up to 3 µg/mL. Comparing the in vitro study results, the authors reported that 20 nm particles were more toxic compared to 80 and 113 nm particles and the potency of silver nanoparticles to induce cell damage is cell type and particle size dependent.

Two studies (oral and inhalation) have evaluated DNA damage in rats. In a 28 day study using the rat bone marrow micronucleus test (OECD TG 474), oral exposure of rats to 30, 300, or 1000 mg/kg bw/day of 60 nm silver particles showed no significant effect on micronucleated polychromatic erythrocytes or bone marrow cells. The authors did not report if silver nanoparticles were found in the bone marrow (Kim et al., 2008).

Exposure of rats to 18 nm silver particles via inhalation at concentrations of 0.7 × 106 particles/cm^3, 1.4 × 106 particles/cm^3, or 2.9 × 106 particles/cm^3, 6 hours/day for 90 days did not induce micronucleated polychromatic erythrocytes or increase the ratio of polychromatic erythrocytes to normal chromatic erythrocytes in bone marrow, indicating no genotoxicity (Kim et al., 2011).

Carcinogenicity

There are no carcinogenicity studies available on silver nanoparticles.

Neurotoxicity

There are three rodent studies investigating the effects of silver nanoparticles on the brain and blood brain barrier function. Rats with 6 hours inhalation exposure to 15 nm silver particles (cumulative dose of 7.2 µg) showed increased silver concentration in the brain and the olfactory region immediately and one day after exposure (Takenaka et al., 2001).

In rats subcutaneously injected with silver nanoparticles (<100 nm) and microparticles (>100 nm) at 62.8 mg/kg bw, silver crossed the blood-brain barrier and accumulated in the brain along with other organs following nanoparticle exposure. The silver content in these organs was significantly higher than in the control group from 8 to 24 weeks after exposure. Increased incidence of astrocyte swelling and neuronal degeneration was reported in rats from 2 to 24 weeks post-exposure due to the accumulation of silver nanoparticles (Tang et al., 2009).

Rats and mice receiving intraperitoneal (50 mg/kg bw), intravenous (30 mg/kg bw) or intracerebroventricular (20 µg) administration of silver nanoparticles (50 – 60 nm) showed significantly altered blood-brain barrier function in regions of the brain. These affected regions also showed pronounced brain edema and decreases in cerebral blood flow. The effects were

more pronounced in mice compared to rats (Sharma et al., 2009).

Cytotoxicity

Several in-vitro studies conducted using various cell lines reported cytotoxicity, oxidative stress, haemolysis or changes in the production of cytokines with exposure to silver nanoparticles.

Cytotoxic effects with exposure to silver nanoparticles have been reported in cultured HeLa cells (Miura and Shinohara, 2009), Raw 264.7 cells (Park et al., 2010), HepG2 human hepatoma cells (Kawata et al., 2009), human A549 lung cancer cell line (Foldbjerg et al., 2009) and human keratinocytes (Samberg et al., 2010).

Silver nanoparticles significantly increased cell death through an oxidative stress related mechanism in mammalian cells (Hsin et al., 2008), LDH leakage and reduced mitochondrial function in mouse C18-4 germline stem cells at a concentration >5 µg/mL (size 15 nm) (Braydich-Stolle et al., 2005) and decreased mitochondrial function along with production of reactive oxygen species in murine neuroblastoma cells at concentrations of 25 µg/mL with 25 nm particles (Schrand et al., 2008).

A significant decrease in mitochondrial function was shown in hepatic cells after single exposures to 15 and 100 nm silver nanoparticles at concentrations ranging from 5-50 µg/mL (Hussain et al., 2005) and in HEKs and fibroblasts after exposure to approximately 15 µg/mL silver nanoparticles extracted from commercially available silver based wound dressings with silver content ranging from 13 to 934 µg/cm^2 (Burd et al., 2007). Decrease in mitochondrial function and onset of apoptosis was reported in human skin carcinoma cells with silver nanoparticles ranging in size from 7-20 nm and at concentrations of 0.78 µg/mL and 1.56 µg/mL respectively (Arora et al., 2008).

One study examined cellular responses from dermal exposure to an antimicrobial gel for wound treatment. Exposure of dermal fibroblasts and primary liver cells to spherical silver nanoparticles (7-20 nm) through the gel did not cause cell death but cellular antioxidant defences were up regulated in both types of cells (Arora, 2009).

Cytokines as mediators of cell immune responses are considered to be good indicators of toxic effects. Changes in the production of many cytokines have been observed from exposure to different concentrations and sizes of silver nanoparticles. Increased production of tumour necrosis factor-α (TNF-α), macrophage inhibitory protein-2 (MIP2) and interleukin 1β (IL-I β) in alveolar macrophages (Carlson, 2008); changes in production of of IL-8 and IL-6 at different concentrations of silver nanoparticles in human mesenchymal stem cells (Greulich et al., 2009) and statistically significant increases in IL-1β, IL-8, TNF-α and IL-6 production in human epidermal keratinocytes and the inhibition of phytohaemagglutinin induced cytokine production in peripheral blood mononuclear cells (Shin et al., 2007) have been reported.

An in vitro assay tested the haemolytic potential of unbound silver particles in human blood to investigate properties contributing to red blood cell damage. Nanoparticles produced higher level of haemolysis compared to micron-sized particles. Particle size and surface area are determined as the key factors that affect haemolysis (Choi et al., 2011).

Commercially available silver nanoparticles were evaluated for cytotoxicity using human dermal and cervical cancer cell lines. The nanoparticles induced elevated levels of oxidative stress, glutathione depletion and damage to cell membranes. Significant differences were observed depending on the cell line which related to their natural antioxidant levels (Mukherjee et al., 2012).

Kim et al. (2012) examined the size-dependent cellular toxicity of silver nanoparticles using particles of three sizes (10, 50 and 100 nm), at doses from 10 to 160 ug/mL and a series of cell lines including mouse osteoblastic MC3T3-E1 cells, human cervical cancer cells and rat adrenal derived PC12 cells. Clear size and dose-dependent cellular toxicity was reported for all tested cell lines. Greater ability to induce apoptosis in MC3T3-E1 cells was seen for 10 nm particles compared to other two sizes tested.

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